FORM PTO-1390 U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 1268-073

U.S. APPLIC. NO. (if known, see 37 CFR 1.5): Not Yet Assigned 09/147346

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IL97/00180	4 June 1997	4 June 1996

TITLE OF INVENTION

CHIMERIC TOXINS FOR TARGETED THERAPY

APPLICANT(S) FOR DO/EO/US

Shai YARKONI, Amotz NECHUSHTAN, Haya LORBERBOUM-GALSKI, and Irina MARIANOVSKI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. [] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. [] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] have been transmitted by the International Bureau.
   c. [] have not been made; however, the time limit for making such amendment has NOT expired.
   d. [] have not been made and will not be made.

8. [] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [] A SECOND or SUBSEQUENT preliminary amendment.

14. [] A substitute specification.

15. [] A change of power of attorney and/or address letter.

16. [X] Other items or information.
    International Preliminary Examination Report with 1 sheet of amended claims.
    International Search Report.
    PCT/IB/308

U.S. APPLIC. NO. (if known, see 37 CFR 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Not Yet Assigned	PCT/IL97/00180	1268-073

	CALCULATIONS	PTO USE ONLY
17. [X] The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $840.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $670.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $760.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $970.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20 -20 =	0	x $18.00	$ 0.00	
Independent Claims	1 -3 =	0	x $78.00	$ 0.00	
Multiple dependent claim(s) (if applicable)			+ $260.00	$ 0.00	
			TOTAL OF ABOVE CALCULATIONS =	$ 840.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 0.00	
			SUBTOTAL =	$ 840.00	
Processing fee of $130.00 for furnishing the English translation later than the [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0.00	
			TOTAL NATIONAL FEE =	$ 840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 0.00	
			TOTAL FEES ENCLOSED =	$ 840.00	
			Amount to be: refunded	$	
			charged	$	

a. [X] A check in the amount of $ 840.00 to cover the above fees is enclosed.

b. [] Please charge my Deposit Account No. in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 07-1337. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Benjamin J. Hauptman
LOWE HAUPTMAN GOPSTEIN & BERNER, LLP
2034 Eisenhower Avenue, Suite 200
Alexandria, VA 22314
(703) 684-1111

Kenneth M. Berner
SIGNATURE

Kenneth M. Berner
NAME

37,093
REGISTRATION NUMBER

December 4, 1998
DATE

BASED ON FORM PTO-1390 (Rev. 5-93)

Applicant or Patentee: Shai YARKONI et al. DR. MEIR NOAM 5135499 Attorney's Docket No.: 1268-073
Serial or Patent No.: 09/147,346
Filed or Issued: December 4, 1998
For: CHIMERIC TOXINS FOR TARGETED THERAPY

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37) CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[ ] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF ORGANIZATION: Yissum Research development Company of the Hebrew University of Jerusalem
ADDRESS OF ORGANIZATION: Jabotinsky Street 46, 91042 Jerusalem, ISRAEL

I hereby declare that the above identified small business concern qualified as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, oral third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled CHIMERIC TOXINS FOR TARGETED THERAPY by inventor(s) Shai YARKONI et al. described in

[] the specification filed herewith.
[X] application Serial No. 09/147,346, filed December 4, 1998.
[] patent no. , issued .

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Mordehai Perlmutter
TITLE IN ORGANIZATION: Managing Director & CEO
ADDRESS OF PERSON SIGNING: 46 Jabotinsky St. POB 4279, Jerusalem 91042, Israel
SIGNATURE:
DATE: January 31, 1999

YISSUM
RESEARCH DEVELOPMENT COMPANY
OF THE
HEBREW UNIVERSITY OF JERUSALEM

Applicant or Patentee: Shai YARKONI et al. Attorney's Docket No.: 1268-073
Serial or Patent No.: 09/147,346
Filed or Issued: December 4, 1998
For: CHIMERIC TOXINS FOR TARGETED THERAPY

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:
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I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled CHIMERIC TOXINS FOR TARGETED THERAPY by inventor(s) Shai YARKONI et al. described in

[ ] the specification filed herewith.
[X] application Serial No. 09/147,346, filed December 4, 1998.
[ ] patent no. , issued .

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NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Mordehai Perlmutter
TITLE IN ORGANIZATION: Managing Director & CEO
ADDRESS OF PERSON SIGNING: 46 Jabotinsky St. POB 4270, Jerusalem 91042, Israel
SIGNATURE:
DATE: January 31, 1999

YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM

Docket No.: 1268-073 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

S, YARKONI

Int'l Appln. No. PCT/IL97/00180

Int'l Filing Date: 4 June 1997

BOX PCT

For: CHIMERIC TOXINS FOR TARGETED THERAPY

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D. C. 20231

Sir:

Preliminary to examination of the above-referenced application, please amend the application:

IN THE CLAIMS:

3. (Amended) Targeted fused chimeric toxins according to claim 1 [wherein the cell targeting moiety is] produced by fusing at the cDNA level an oligonucleotide encoding ten amino acids of a gonadotropin releasing hormone analog [and the killing moiety consists of] to a mutated [form] sequence of the full length Pseudomonas Exotoxin, [providing] encoding the protein GnRH-PE66.

4. (Amended) Targeted fused chimeric toxins according to claim 1 [wherein the cell targeting moiety is] produced by fusing at the cDNA level an oligonucleotide encoding ten amino acids of a gonadotropin releasing hormone analog to a sequence [and the killing moiety consists] consisting of domains II and III of the Pseudomonas Exotoxin, [providing] encoding the protein GnRH-PE40.

Claim 5, please delete "and 3".

Claim 6, please delete "and 4".

Claim 7, please change "claims 1-4" to --claim 1--.

Please delete claim 8.

Claim 9, please change "claims 1-4 and 7" to --claim 1--.

Claim 11, please change "claims 1-4 and 7" to --claim 1--.

Claim 13, please change "claims 1-4 and 7" to --claim 1--.

Claim 15, please change "claims 1-4 and 7" to --claim 1--.

Claim 17, please change "claims 1-4 and 7" to --claim 1--.

Claim 19, please change "claims 1-4 and 7" to --claim 1--.

Please add the following new claim:

--21. A plasmid comprising a promoter operably linked to a DNA molecule encoding a targeted fused chimeric toxin as defined in claim 1.

REMARKS

The above-referenced application is amended to improve syntax and to delete the multiple dependency of claims 5, 6, 8, 9, 11, 13, 15, 17, & 19 to avoid the multiple dependent claim filing fee.

Respectfully submitted,

LOWE HAUPTMAN GOPSTEIN GILMAN & BERNER, LLP

Kenneth M. Berner

Kenneth M. Berner
Registration No. 37,093

2034 Eisenhower Avenue, Suite 200
Alexandria, Virginia 22314
(703) 684-1111 KMB:leb
Facsimile: 703-518-5499

CHIMERIC TOXINS FOR TARGETED THERAPY

Field of the invention

The present invention relates generally to therapeutic agents useful particularly in cancer targeted therapy but also in treating malignant carcinomas such as breast, colon, hepatic, ovarian and renal carcinomas and treating benign tumors of the uterus, hyperplasia, endometriosis, BPH, polycystic disease of the breast and pituitary adenomas.

More specifically the said invention relates to Pseudomonas Exotoxin based chimeric toxins aimed at those neoplastic cells bearing gonadotropin releasing hormone binding sites. The present invention further relates to pharmaceutical compositions comprising as an active ingredient the above mentioned neoplastic cell targeted chimeric toxins. Furthermore the present invention relates to a method for the production of said chimeric toxins. These chimeric proteins, according to the present invention, are comprised of cell targeting moieties which consist of gonadotropin releasing hormone homologues linked to cell killing moieties which consist, preferably, of the bacterial toxin Pseudomonas Exotoxin A , for recognizing and destroying neoplastic cells bearing gonadotropin releasing hormone binding sites.

Targeting is a term for the selective delivery of chemotherapeutic agents to specific cell populations. It is possible to create chimeric molecules that possess cell targeting and cellular toxin domains. These chimeric molecules function as cell selective poisons by virtue of their abilities to target selective cells and then kill those cells via their toxin component. Pseudomonas Exotoxin A (hereinafter called PE), a bacterial toxin used in construction of such chimeric proteins, acts by irreversibly arresting protein synthesis in eukaryotic cells, resulting in

cell death.

The term "gonadotropin releasing hormone homologues" in this invention relates to the gonadotropin releasing hormone gene itself or its analogues and antagonists.
Also included in the scope of the present invention are salts of the described chimeric proteins. The term "salts" includes both salts of carboxy groups as well as acid addition salts of amino groups of the protein molecule. The invention further relates to pharmaceutical compositions comprising the chimeric proteins as defined above together with a pharmaceutically acceptable inert carrier. The proteins of the present invention may be administered by methods known in the art for the administeration of proteins.

Background of the invention

Gonadotropin releasing hormone (hereinafter called GnRH) participates in the hypothalamic - pituitary gonadal control of human reproduction. The involvment of GnRH has been demonstrated in several carcinomas and GnRH analogue treatment has been applied in breast, prostatic, pancreatic, endometrial and ovarian cancers (Kadar T. et al. Prostate 12: 229 - 307, 1988). These analogues suppress tumor cell growth in vitro and in vivo. The existence of GnRH binding sites was revealed in the corresponding malignant cells and in well established cell lines (Emons G. et al. J.Clin.Endocrinol.Metab. 77: 1458 - 1464, 1993), though prelimenary results suggest that the GnRH receptor involved may differ from the previously documented receptor (Kadar et al. Biochem. Biophs. Res. Comm. 189: 289 - 295, 1992).

Although GnRH binding sites have been demonstrated in a number of solid tumors and various carcinoma cell lines derived mainly from hormone dependant tissues, their existence in colon or renal carcinoma has not been previously documented. The presence of specific GnRH binding sites in colon, breast, prostate, ovarian endometrium, renal

and liver carcinomas, is shown here. Surprisingly, the specific GnRH binding sites are not limited to hormone-dependant tissues, as indicated by the marked killing of colon carcinoma, renal cell carcinoma and hepatocarcinoma cells.

WO Patent No. WO93/15751 describes various conjugates of GnRH, a linking group and Pseudomonas Toxin A, prepared using the techniques of synthetic organic chemistry, used for the sterilization of animals by killing gonadotrophin releasing cells of the animals pituitary gland.

The present invention describes the construction, by the techniques of genetic enginering, of PE based chimeric toxins, aimed at targeting those neoplastic cells bearing GnRH binding sites. The chimeric toxins of the present invention are fusion proteins and, as such, do not contain a chemical linking group (as in the above mentioned patent). Therefor, they are completely different proteins from the molecules described in the WO patent.

Using different kinds of targeting moieties, a large number of immunotoxins have been generated in the last 20 years by chemical linkage techniques or recombinant DNA technology. The size of these targeting moieties varies widely, ranging from large antibodies to small growth factors, cytokines and antibody fragments.

The ability of large chimeric proteins, as the GnRH-PE constructions described in the present invention, to target cells via a very small portion of the polypeptide (a peptide of ten amino acids, as used as the targeting moiety of the present invention), and yet retain their original functions, namely binding and internalization, open up new possibilities in designing targeted immunotoxins.

Colon, breast, and prostate cancer - three out of the four major malignancies occuring in humans, together with ovarian, endometrium, renal and liver carcinomas, account for more than 50% of cancer related death. The presence of specific GnRH binding sites in all these cancers, may suggest a more general role of GnRH and/or GnRH - like

peptides in the malignant process.

Collectively, these results disclosed what could be considered the Achilles' heel of these malignant growths, a finding which could open up new vistas in the fight against cancer.

In view of their efficient growth inhibition of the above mentioned cancer cells and their specificity regarding the non target cells, the novel GnRH-PE chimeric toxins are promising candidates for cancer treatment.

Summary of the invention

The present invention relates particularly to neoplastic cells targeted chimeric toxins comprising of cell targeting moieties and cell killing moieties for recognizing and for destroying the neoplastic cells, wherein the cell targeting moieties consist of gonadotropin releasing hormone homologues and the cell killing moieties consist of Pseudomonas Exotoxin A. The present invention further relates to pharmaceutical compositions containing as an active ingredient these neoplastic cells targeted chimeric toxins and to a method for the production of these chimeric toxins. The said invention also relates to a method for cancer therapy, treating malignant carcinoma cells and benign hyperplasia including uterine lyomyoma cells, extra uterian endometrial island cells, benign hyperplasia of prostate and breast and pituitary tunor adenoma cells, by the use of the above mentioned chimeric toxins.

Detailed description of the invention

The present invention describes Pseudomonas Exotoxin A (PE) based chimeric toxins constructed by ligating an oligonucleotide encoding ten amino acids of a gonadotropin releasing hormone (GnRH) analog (GnRH coding sequence with tryptophane replacing glycine as the sixth amino acid) upstream to a mutated form of PE (domains I(mutated),II and III) thereby generating GnRH-PE66, and a ten amino acid synthetic GnRH oligomer(GnRH coding sequence with tryptophane replacing glycine as the sixth amino acid) ligated to domains II and III of the PE, thereby generating GnRH-PE40 protein.

The applications, potential markets and commercial advantages of the said chimeric proteins according to the present invention are listed:

These are two main applications:

1) The malignant carcinomas:

Breast, colon, hepatic, ovarian and renal carcinomas were all sensitive to GnRH - PE mediated cytotoxicity. Thus, the potential market for this new chimeric protein includes all carcinoma patients either as a first line of treatment or for patients in which other modalities of treatment had failed.

2) The benign tumors of the uterus and hyperplasia:

This group of pathalogies includes various tissues that are known to be sensitive to GnRH and thus can be targeted by the GnRH-PE chimeric proteins.

a. Uterine - Uterine lyomyoma is the most common benign tumor in women. The uterine myomas are found to carry a large number of GnRH receptors. GnRH analogs are clinically used for down regulation and shrinkage of these myomas. The disadvantage of GnRH analogs is that these compounds can not be used for long periods and the myomas return to their original size after cessation of the treatment. The use of GnRH-PE for the destruction of the myomas can help to avoid what was considered to be iminant hysterectomies, as well as hemostatic drugs taken by these patients. The optional market includes women with fibroid uterus.

b. Endometriosis - Endometrioma: The existence of endometrial tissue out of the uterus leads to the disease called endometriosis which can cause infertility, abdominal pain and even surgical emergencies.
The endometrial islands are known to be very sensitive to hormonal changes. One of the therapeutic modalities found to be clinically effecient is the GnRH analog. Using GnRH-PE the endometrial activity of these islands can be arrested, thereby helping infertile couples as well as women who are undergoing laparotomy for the resection of these endometrial islands. The treatment of both the lyomyoma and the endometria can be administered systematically or locally by either ultra sonic or laparoscopic guided injection into the endometriomal peritoneal cavity or by a slow release into the uterine cavity.

c. Benign Prostatic Hyperplasia (BPH):
The prostatic cells are known to express GnRH receptors and prostatic cancer is successfully treated today with GnRH agonists. The BPH cause severe symptoms of dissuria urinary retention and sometimes can be treated only by prostatectomy. The use of GnRH-PE can therefor replace all prostatectomies procedures carried on prostate hyperplasia that is not malignant.
The potential market is all the elderly men suffering from symptomatic prostate enlargement. GnRH-PE chimeric proteins may be administered systemically or trans uterally.

d. Polycystic disease of the breast:
The mammary cells are also known to express the GnRH receptors. As in the case of BPH, the polycystic disease of the breast may be symptomatic, cause pain and may mimic breast carcinoma. The use of GnRH-PE may eliminate the need for numerous check-ups and needless mamographies and help woman suffering from breast pains and ***** of breast malignancy.
Thhe potential market is a large population of woman in whom polycystic breast disease is diagnosed. GnRH-PE may be administered systemically.

e. Pituitary adenoma: some of the pituitary adenomas are derived from gonadotropic cells. The pituitary adenoma, even though non malignant, can cause a grave prognosis by causing local pressure on vital organs (eyes, brain stem). The trans-sphenoidal surgery used for the pituitary adenoma has many disadvantages including recurrency and neurological sequella. GnRH-PE may be aimed directly against the gonadotropic cells without damaging other functions of the pituitary gland. GnRH-PE chimeric toxin may be administered intra-thecally.

Commercial advantages:

1. The wide variety of tumors that respond to the GnRH-PE chimeric protein.
2. The high selectivity that allows a large therapeutic range.
3. The use of GnRH as a targeting peptide leaving the large population of postmenopausal women in whom the GnRH has no physiological role perfect candidates for the treatment.
4. Its' high specificity enables the systemic administration together with the local effect.
5. The ability to eradicate small populations of cells in a tissue that will not be harmed by itself.

The proteins of the present invention may be administered by methods known in the art for the administeration of proteins. Also included in the scope of the present invention are salts of the described chimeric proteins. The term "salts" includes both salts of carboxy groups as well as acid addition salts of amino groups of the protein molecule. Salts of the carboxy group may be formed by methods known in the art and include both inorganic salts as well as salts with organic bases. The invention further relates to pharmaceutical compositions comprising the chimeric proteins as defined above together with a pharmaceutically acceptable inert carrier. The pharmaceutical composition may be administered by injection (intra-veneous, intra-articular,

sub-cutaneous, intra-muscular, intra-peritoneal) toppical application, oral administration, sustained release, or by any other route including the enteral route.

The said invention will be further described in detail by the following experiments and figures. These experiments and figures do not intend to limit the scope of the invention but to demonstrate and clarify it only.

Description of the Figures:

Figure 1: Construction and expression of the GnRH-PE66 chimeric toxin. A, SDS-PAGE gel and B, immunoblotting analysis of TGnRH-PE66 plasmid expression. Whole cell extract of the lysed bacteria (lane 1). Soluble fraction (lane 2). Insoluble fraction (lane 3). C, construction of TGnRH-PE66 plasmid.

T7 promotor. GnRH analogue peptide. Ampicillin^R

PE664glu. The numbers represent the corresponding amino acids.

Figure 2: The effect of increasing concentrations of GnRH-PE66 on various cell lines.

A: SW-48 colon carcinoma, HepG2 hepatocarcinoma, Caco2 colon carcinoma.

B: OVCAR3 ovarian carcinoma, Hela cervix adenocarcinoma, MDA breast carcinoma, HT-29 colon carcinoma.

Figure 3: The effect of GnRH-PE66 on various primary cultures. A, colon carcinoma primary cultures established from three patients. B, renal cell carcinoma primary culture. C, breast carcinoma primary cultures established from four patients. D, ovarian carcinoma primary cultures established from two patients. E, metastases primary cultures established from the corresponding patients represented in A,C and D by the same symbols. ● colon carcinoma metastases. ♣ breast carcinoma metastases. ✠, ◇ two ovarian carcinoma metastases. F, control cells: # leukocytes. ▶bonemarrow. • fibroblasts. ▽colon.

Figure 4: Histopathological diagnosis of primary cultures. A, anti-keratin positive staining of a colon primary culture. B, anti-desmin negative staining of a colon primary culture.

Figure 5: Displacement of [^{125}I] GnRH bound to membranes of SW-48 cells by: ○ GnRH-PE66. ■ GnRH analogue (des-Gly^{10},[d-Ala^{6}]-LHRH).

Figure 6: Purification of GnRH-PE66.

1 - protein marker. 2 - whole cell extract. 3 - soluble fraction. 4 - insoluble fraction after refolding. 5 - after DEAE - Sepharose column. 6 - after Sepharyl S-200 HR column.

Figure 7: Purification of GnRH-PE40:

1 - protein marker. 2 - whole cell extract. 3 - soluble fraction. 4 - insoluble fraction after refolding. 5 - after DEAE - Sepharose column. 6 - after Sepharyl S-200 HR column.

Figure 8: Effects of GnRH-PE chimeric proteins on SW-48 colon carcinoma cell line:

● GnRH-PE66 insoluble fraction after refolding.

▲ GnRH-PE66 purified protein.

● GnRH-PE40 purified protein.

Experiments

1. GnRH-PE66 chimeric toxin construction

A plasmid vector carrying the mutated full length PE gene (pJY3A1136-1,3) (Chandhary V., Jinno Y., Gall M., Fitzgerald D. and Patsan T. J. Biol. Chem. 256, 16306-16310, 1990) was cut with NdeI and Hind III. The insert was a 36 base pair synthetic oligomer consisting of the GnRH coding sequence with tryptophan replacing glycin as the sixth amino acid, was flanked by NdeI (5' end) and HindIII (3' end) restriction sites. The resulting TGnRH-PE66 plasmid was confirmed by restriction endonucleases digestion and DNA sequence analysis (Figure 1c).

2. TGnRH-PE40 plasmid construction

To construct the GnRH-PE40 protein (GnRH-domains II and III of the PE), the TGnRH-PE66 plasmid vector (fig. 1c) was digested with NdeI and BamHI and ligated to a NdeI-BamHI 750bp fragment from the plasmid PHL-906 (Fishman A., Bar-Kana Y., Steinberger I. and Lorberboum-Galski H. Biochemistry 33, 6235-6243, 1994) along with an insert which is a 36 base pair synthetic oligomer consisting of the GnRH coding sequence with tryptophan replacing glycin as the sixth amino acid, flanked by NdeI (5' end) and HindIII (3' end) restriction sites. The resulting TGnRH-PE40 plasmid was confirmed by restriction endonucleases digestion and DNA sequence analysis.

3. Protein expression

Protein expression method was the same for GnRH-PE40 and GnRH-PE66, unless mentioned. Escherichia coli strain BL21 (DE3) carrying the plasmid TGnRH-PE66 was grown in LB medium contai`ning ampicillin (100µg/ml) and Escherichia coli strain BL21 (DE3) carrying the plasmid GnRH-PE40 was grown in super LB medium containing ampicilin (50 µg/ml). After reaching an A600 value of 1.5 - 1.7, the cultures were induced 90 minutes for GnRH-PE66 and over night for GnRH-PE40, at 37°C with 1 mM isopropyl-d-thiogalactoside (IPTG). Cells were collected by centrifugation and the pellet was incubated at -70°C for several hours.

The frozen pellet was thawed and suspended in lysis buffer (50mM Tris HCl, pH 8.0, 1mM EDTA and lysozyme 0.2 mg/ml), followed by sonication (3 x 30 seconds) and centrifugation at 35,000 x g for 30 minutes. The supernatant (soluble fraction) was removed and the pellet (insoluble fraction) served as the source for the chimeric proteins and for their purification.

Analysis of the insoluble fraction by SDS/PAGE gel electrophoresis revealed a major band (70%) with an expected molecular mass of 67kDa, corresponding to the chimeric protein, and two major unrelated bacterial proteins of 42 and 28 kDa (fig. 1a). Immunoblotting with polyclonal antibodies against PE, confirmed these data (fig. 1.b).

4. Effect of the GnRH-PE66 chimeric proteins on various cell lines

In the experiments described below, the insoluble fraction of E.coli expressing cells was used as the source of the GnRH-PE66 chimeric protein.

The cytotoxic activity of GnRH-PE66 was tested in various established cell lines: SW-48 colon carcinoma, HepG2 hepatocarcinoma, Caco2 colon carcinoma, OVCAR3 ovarian carcinoma, Hela cervix adenocarcinoma, MDA breast

carcinoma, HT- 29 colon carcinoma. Unless specified, all cell lines were maintained in RPMI 1640 medium, cultured in 100mm petri dishes in a humidified atmosphere of 5%CO2 / 95% air at 37°C. HepG2 and Caco2 were maintained in Eagle's Minimal Essential Medium, Hela cells were maintained in Dulbecco's Modified Eagle's Medium. All media were supplemented with 10% fetal calf serum, 2mM L-glutamin, 100 units/ml of penicillin and 100µg/ml streptomycin. On day 0, cells (10^4 in 0.2 ml culture medium) were seeded in 96 well tissue culture microplates and 24 hours later various concentrations of the GnRH-PE66 were added. After 24 hours incubation [^{3}H]leucine [5µCi per well] was added for an additional 24 hours. At day 3, the plates were stored at -70°C for several hours, followed by a quick thawing at 37°C. Cells were harvested on filters and the incorporated radioactivity was measured with a beta counter. The chimeric protein was found to kill cells in a dose-dependent manner, with considerable variation between cell lines (table 1) ranging from the strong response of HepG2 hepatocarcinoma, SW-48 and Caca2 colon carcinomas (figure 2a) to the intermediate one of ovarian carcinoma OVCAR3, breast carcinoma MDA MB-231, colon carcinoma HT-29 and cervix adenocarcinoma Hela (figure 2b). Although cytotoxicity was measured by inhibition of amino acid incorporation, cell death was reflected in cell number and\or cell necrosis 24 hours following the addition of the chimeric protein.

To confirm the specificity of GnRH-PE66 activity, two other PE based recombinant proteins, expressed and extracted under the same conditions, were used as controls. No substantial growth inhibition was exerted by either PE664Glu, encoded by the mutated full length PE gene, or by PIS2, an unrelated 80 bp sequence fused to PE664Glu. When 15 µg/ml of PE664Glu or PIS2 were added, protein synthesis ranged from a slight increase to 20% inhibition in the different cultures. Growth inhibition resulting from treatment with one of the two proteins was considered nonspecific.

The results are expressed as the percent of the control experiments in which cells were not exposed to any protein (results are summarized in table 1 and in fig. 5).

Table 1: Cytotoxic activity of GnRH-PE66 on various cell lines.

Cell line	Origin	ID_{50} (µg total protein/well)*
Caco2	Colon carcinoma	0.4
HT-29	Colon carcinoma	1.2
SW-48	Colon carcinoma	0.3
OVCAR3	Ovarian carcinoma	3
MDA MB-231	Breast carcinoma	2.3
Hela	Cervix adenocarcinoma	1.8
HepG2	Hepatocarcinoma	0.3

* The ID_{50} values show the effect of the insoluble fraction enriched with the chimeric protein.

5. The effect of GnRH-PE66 on various primary cultures

In order to evaluate the cytotoxic effectiveness of the chimeric proteins on cells resembling the original in vivo tumors as closely as possible and to exclude the possibility that the GnRH-PE66 cytoxicity was a characteristic developed by cells upon prolonged passages, primary cultures were established.

Fresh tissue specimens were obtained from various cancer patients undergoing therapeutic debulking procedures. Control specimens were obtained from donors or patients undergoing diagnostic or therapeutic procedures for non - malignant diseases. All tissue specimens were washed several

times with Leibovitz (L15) medium, and extensively cut with a scalpel. The preparations were then enzymatically proteolysed for 2 hours at 37°C with gentle shaking in Leibovitz medium containing collagenase type I (200u/ml), hyaluronidase (100u/ml), penicillin (1000units/ml), streptomycin (1mg/ml), amphotericin B (2.5 µg/ml) and gentamycin (80 µg/ml). Tissue preparations were centrifuged 10 minutes at 200 x g and the pellets were suspended in RPMI 1640 medium, supplemented with 10% fetal calf serum, penicillin (100u/ml) and streptomycin (100µg/ml) and plated in 100mm petri dishes. Cells were grown for one to three weeks to a density of 8 x 10^6 cells and histopathological diagnoses and cytotoxic assays were performed. Normal leukocytes from peripheral blood and bone marrow aspirates for cytotoxic assays were obtained by diluting whole blood in one volume of phosphate - buffered saline. The diluted sample was placed over an equal volume of Ficoll - Paque and centrefuged for 10 minutes at 200 x g. The cells were resuspended and plated in RPMI 1640 medium containing 20% fetal calf serum, 4 mM l - glutamine, 50 µM ß mercaptoethanol, non essential amino acids, 1mM sodium pyruvate, penicillin (100 units/ml) and streptomycin (100µg/ml).

The cytotoxic effect of the chimeric protein was variable (table 2) with up to three-fold differences in ID50 observed in colon, breast and ovarian primary cultures originated from different patients (figures 3a,c and d respectively).

Table 2: Cytotoxic effect of GnRH-PE66 on various primary cultures

Origin	ID_{50} (µg total protein/well)[a]
Colon carcinoma	0.8 - 2.5[b]
Renal cell carcinoma	1.2
Breast carcinoma	1 - 3[c]
Ovarian carcinoma	1.6 - 3[d]
Bladder carcinoma	no effect
Control cells:	
Colon	no effect[e]
Fibroblasts	" "
Bone marrow	" "
Leukocytes	" "

[a] The ID_{50} values show the effect of the insoluble fraction enriched with the chimeric protein

[b] n=3 [c] n=4 [d] n=2

[e] increasing concentration of GnRH-PE66 did not affect cell growth

In cases where metastasis biopsies could also be obtained, cultures of primary tumors alongside with the metastasis were examined for GnRH-PE66 cytotoxicity. The metastatic cells responded in the same manner, and their ID_{50} were even lower than those of the primary tumors. This may be explained by the high homogeneity of the metastasis culture compared with that of the primary culture.

GnRH-PE66 was also tested on cultures of benign colon peripheral blood bone marrow and skin fibroblasts from healthy donors. The addition of up to 15µg/ml of the

chimeric protein did not result in any measurable dose dependant killing (fig 3f).

6. Histopathological diagnosis of primary cultures

One of the basic questions regarding the veracity of the primary cultures assays is of the epithelial origin of the cells. The tendency of cells in primary culture to lose their epithelial morphology has been described in several carcinomas. To confirm the absence of any substantial amount of "contaminating" fibroblasts, differential staining was performed.

Cells were stained as follows: 10,000 cells were plated on a microscope slide using a cytospin, followed by several minutes incubation at room temperature. Dried slides were fixed by soaking in -20°C cold methanol for 15 minutes and in -20°C cold acetone for a few seconds. Slides were kept at -20°C until staining. Staining was carried out with anti-desmin and anti-keratin antibodies to distinguish fibroblast from epithelial cells, respectively. This staining indicated that the vast majority of the cells a980-100%) were indeed epithelial, even in cases where the cultures exhibited a fibroblast-like shape (fig 4).

Further confirmation was achieved by staining with specific anti tumor marker antigens according to the type of cancer. Formalin fixed sections from the original tumors and the primary cultures cells displayed the same pattern and intensity of staining.

7. Specific binding by GnRH-PE66

To support the findings that colon carcinoma cell lines and primary cultures can be targeted and killed by GnRH-PE66, the ability of plasma membrane fractions from a colon carcinoma cell line to specifically bind GnRH, was examined. The addition of increasing concentrations of GnRH-PE66 chimeric toxin resulted in dose - related displacement of the ^{125}I -

GnRH bound to these membranes. A semiconfluent 100mm dish of the SW-48 colon carcinoma cell line was washed and the cells were scraped off the plate with a rubber policeman. The collected cells were homogenized in ice - cold assay buffer (10mM Tris - HCl, pH 7.6, 1mM dithiothreitol, 0.15% bovine serum albumin, 1mM EDTA) and centrifuged at 250 x g for 15 minutes (4°C). The resulting pellet was discarded and the supernatant was centrifuged at 20,000 x g for 30 minutes (4°C). The plasma membrane pellet was resuspended in cold assay buffer. Aliquots containing 70 µg plasma membrane protein in a final volume of 100µl, were incubated for 2 hours on ice with 6 x 10^{-6}M (240,000 cpm) ^{125}I-GnRH either in the presence or absence of (10^{-4} - 10^{-10}M) unlabeled GnRH authentic peptide and analog (des - Gly,[d-Ala]-LHRH) or (2.5 x 10^{-5} - 10^{-9}M) GnRH-PE66 chimeric toxin. Following incubation, samples were washed through Whatman GF/C filters with 10 ml of cold assay buffer and counted in a gamma counter.

The addition of increasing concentrations of GnRH-PE66 chimeric toxin resulted in dose related displacement of the ^{125}I-GnRH bound to these membranes. Unlabeled authentic GnRH peptide and the analogue des-Gly10 [D-Ala6]-LHRH produced similar results. As can be seen in figure 5, binding of the labeled GnRH to SW-48 colon carcinoma cell line was specific and displacement by the GnRH-PE66 chimeric toxin was as efficient as that by the GnRH analogue peptide. There was 37% non specific binding.

8. GnRH-PE40 and GnRH-PE66 purification

The pellet of the insoluble fraction was suspended and stirred on ice in denaturation buffer (6M guanidium HCl, 0.1 M Tris HCl, pH 8.6 1mM EDTA 0.05M NaCl and 10mM DDT). After an additional centrifugation, the reduced and denatured protein was diluted 1:100 in refolding buffer (50mM Tris HCl, pH 8, 1mM EDTA, 0.25M NaCl, 0.25 M L-arginine and 5mM DTT) and kept at 4°C for 48 hours. Refolded protein

solutions were diluted to 8 mS in TE20 buffer (20mM Tris pH 8.0, 1mM EDTA). DEAE Sepharose was added and stirred for half an hour at 4°C before being packed onto a column. Washing of the column was done with 80mM NaCl, in TE20 buffer for GnRH-PE66 and 50mM NaCl in TE20 buffer for GnRH-PE40. Elution was preformed with the linear gradient of 2 x 200ml of 0.08 - 0.35M NaCl, in TE20 (20mM Tris pH 8.0, 1mM EDTA) buffer. The peak fractions were pooled, 0.5M L-arginine was added and stirred cell was used for concentration. (fig. 2 and fig. 3). 3ml of the pooled fractions from the ion exchange column were loaded onto a Sephacryl S-200 HR gel filtration column, in 0.5M NaCl, 0.15M K -phosphate buffer, pH 6.0. The peak fractions were pooled, dialyzed against phosphate saline buffer and kept in aliquotes at -20°C. Purification of GnRH-PE66 and GnRH-PE40 is demonstrated in figures 6 and 7 respectively.

9. Effect of highly purified GnRH-PE chimeric proteins on SW-48 colon carcinoma cell line

The cytotoxic activity of the purified GnRH-PE66 and GnRH-PE40 on SW-48 colon carcinoma cell line was assessed by measuring the inhibition of protein synthesis. The chimeric proteins were found to kill cells in a dose dependent manner. The ID_{50} of the purified GnRH-PE66 chimeric toxin was two to three times lower than the refolded insoluble fraction. The ID_{50} of the GnRH-PE40 purified protein was three to four times lower than the purified GnRH-PE66 (fig. 8).

AMENDED

Claims

1. Targeted fused chimeric toxins produced by genetic engineering techniques, comprising of cell targeting moieties and cell killing moieties for recognizing and for destroying specific cells bearing gonadotropin releasing hormone binding sites, wherein the cell targeting moieties consist of gonadotropin releasing hormone and the cell killing moieties consist of a cell killing Toxin.

2. Targeted fused chimeric toxins according to claim 1 wherein the specific cells bearing gonadotropin releasing hormone binding sites are selected from malignant adenocarcinoma cells, benign uterine lyomyoma cells, endometrial island cells and pituitary tumor adenoma cells.

3. Targeted fused chimeric toxins according to claim 1 wherein the cell targeting moiety is an oligonucleotide encoding ten amino acids of a gonadotropin releasing hormone analog fused at the cDNA level to the killing moiety that consists of a mutated form of the full length Pseudomonas Exotoxin, encoding the protein GnRH-PE66.

4. Targeted fused chimeric toxins according to claim 1 wherein the cell targeting moiety is an oligonucleotide encoding ten amino acids of a gonadotropin releasing hormone analog fused at the cDNA level to the killing moiety that consists of domains II and III of the Pseudomonas Exotoxin, encoding the protein GnRH-PE40.

5. A method for the production of targeted fused chimeric toxin GnRH-PE66 as defined in claim 1 and 3 comprising fusing an oligonucleotide encoding ten amino acids of a gonadotropin releasing hormone analog upstream to a mutated form of PE.

6. A method for the production of cancer cell targeted chimeric toxin GnRH-PE40 as defined in claim 1 and 4 comprising ligating an oligonucleotide encoding ten amino acids of a gonadotropin releasing hormone analog upstream to domains II and III of the PE.

7. Pharmaceutical composition useful for treatment in cancer therapy comprising as active ingredients chimeric toxins as defined in claims 1-4.

8. Chimeric toxins as defined in claims 1-4 and pharmaceutical compositions as defined in claim 7 containing the same as defined in claim 7 for use in the treatment of malignant carcinomas and benign tumors.

9. A method for cancer therapy in mammals by administering to the patient`s body chimeric toxins or their pharmaceutical compositions as defined in claims 1 - 4 and 7.

10. A method for cancer therapy according to claim 9 wherein the chimeric toxins are administered by systemic administration or by trans cervical washing of the endometrial cavity.

11. A method for treating endometriosis by administering chimeric toxins or their pharmaceutical compositions as defined in claims 1 - 4 and 7 to the patient`s body.

12. A method for treating endometriosis according to claim 11 wherein the chimeric toxins are administered by peritoneal washings or by ultrasonic guided or laparoscopic intra-endometrial injections or by systemic administeration.

13. A method for treating uterine myomas by administering chimeric toxins or their pharmaceutical compositions as defined in claims 1 - 4 and 7 to the patient`s body.

14. A method for treating uterine myomas according to claim 13 wherein the chimeric toxins are administered by trans cervical washing of the endometrial cavity.

15. A method for treating pituitary adenomas by administering chimeric toxins or their pharmaceutical compositions as defined in claims 1 - 4 and 7 to the patient`s body.

16. A method for treating pituitary adenomas according to claim 15 wherein the chimeric toxins are administered intra- thecally.

17. A method for treating BPH by administering chimeric toxins or their pharmaceutical compositions as defined in claims 1 - 4 and 7 to the patient's body.

18. A method for treating BPH according to claim 17 wherein the chimeric toxins are administeres systemically or by trans - uteral administration of GnRH-PE.

19. A method for treating polycystic breast disease by administering chimeric toxins or their pharmaceutical compositions as defined in claims 1 - 4 and 7 to the patient's body.

20. A method for treating polycystic breast disease according to claim 19 wherein the chimeric toxins are systemically administered.

Figure 1A

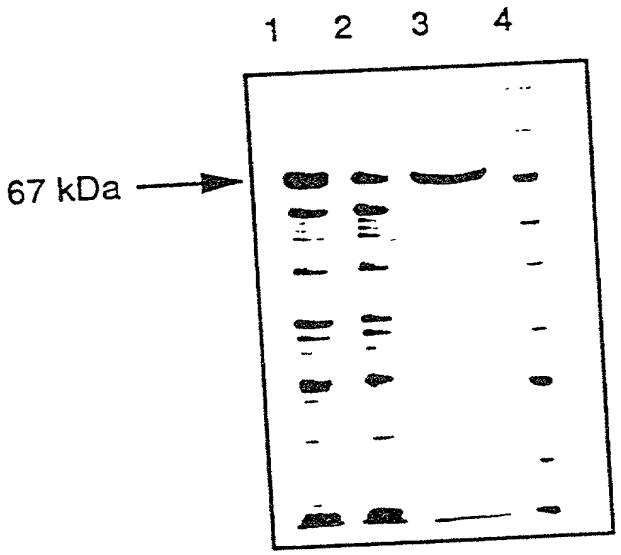


Figure 1B

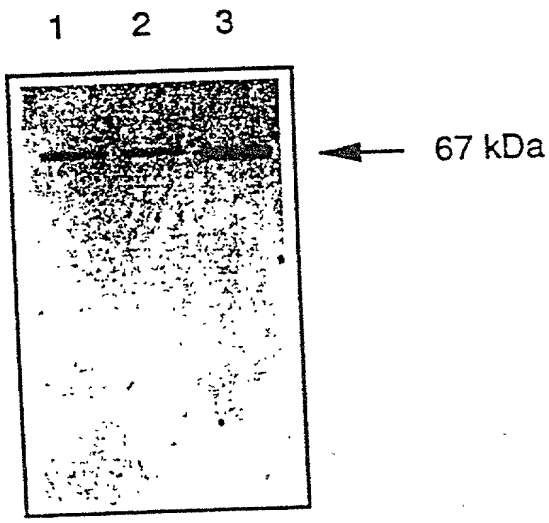


Figure 1C

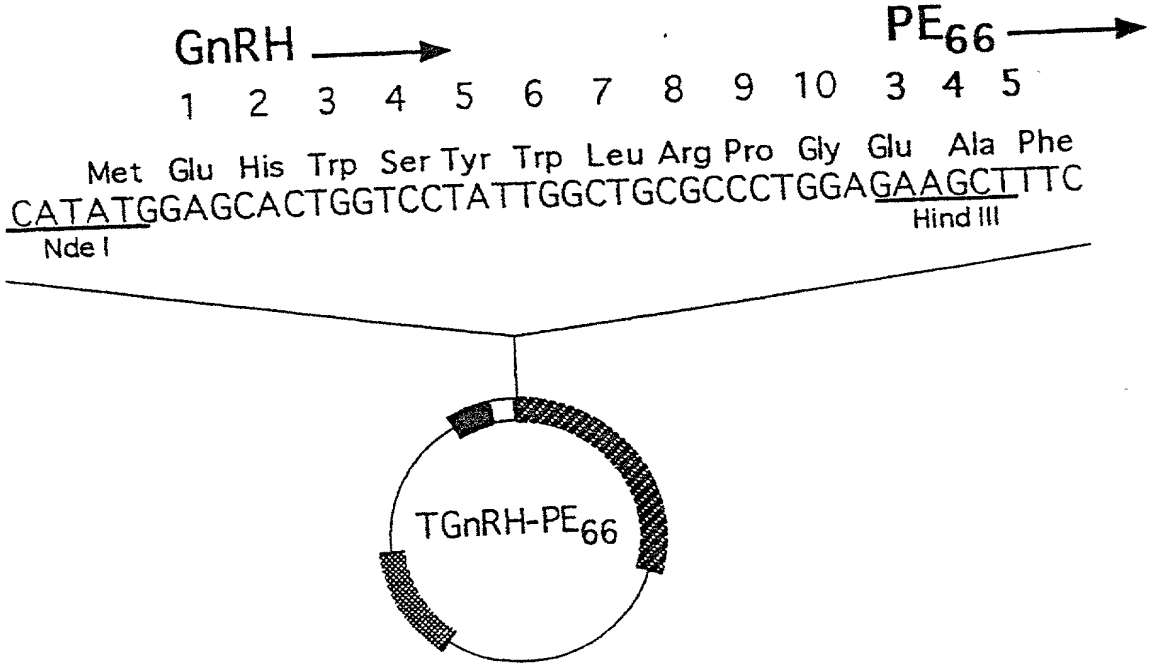


Figure 2A

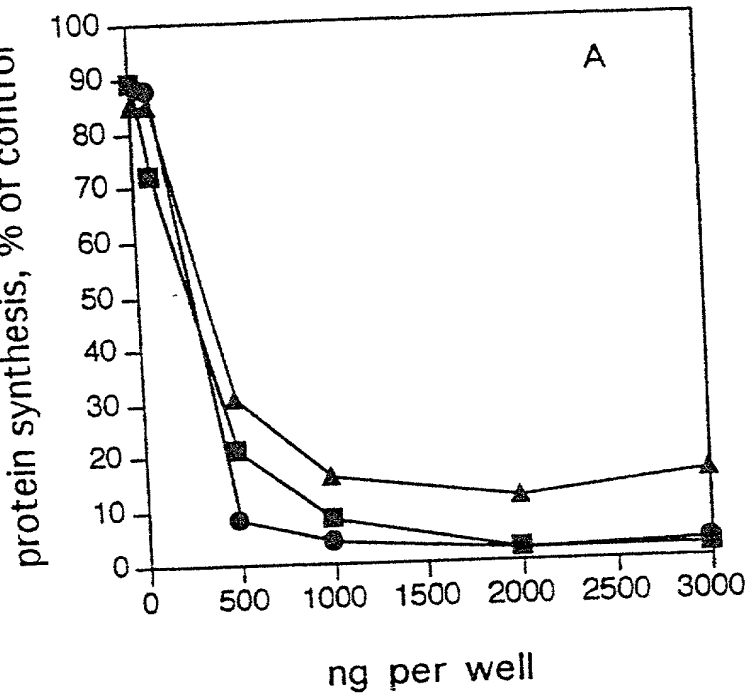
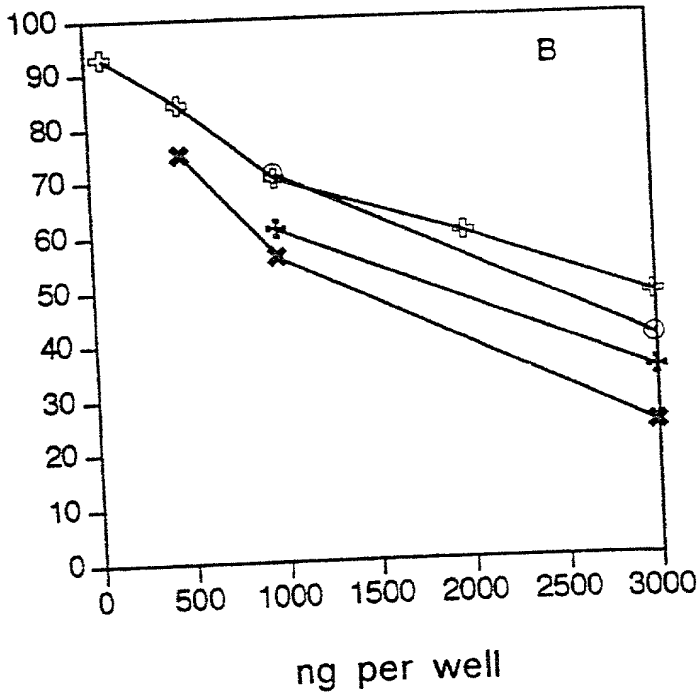


Figure 2B



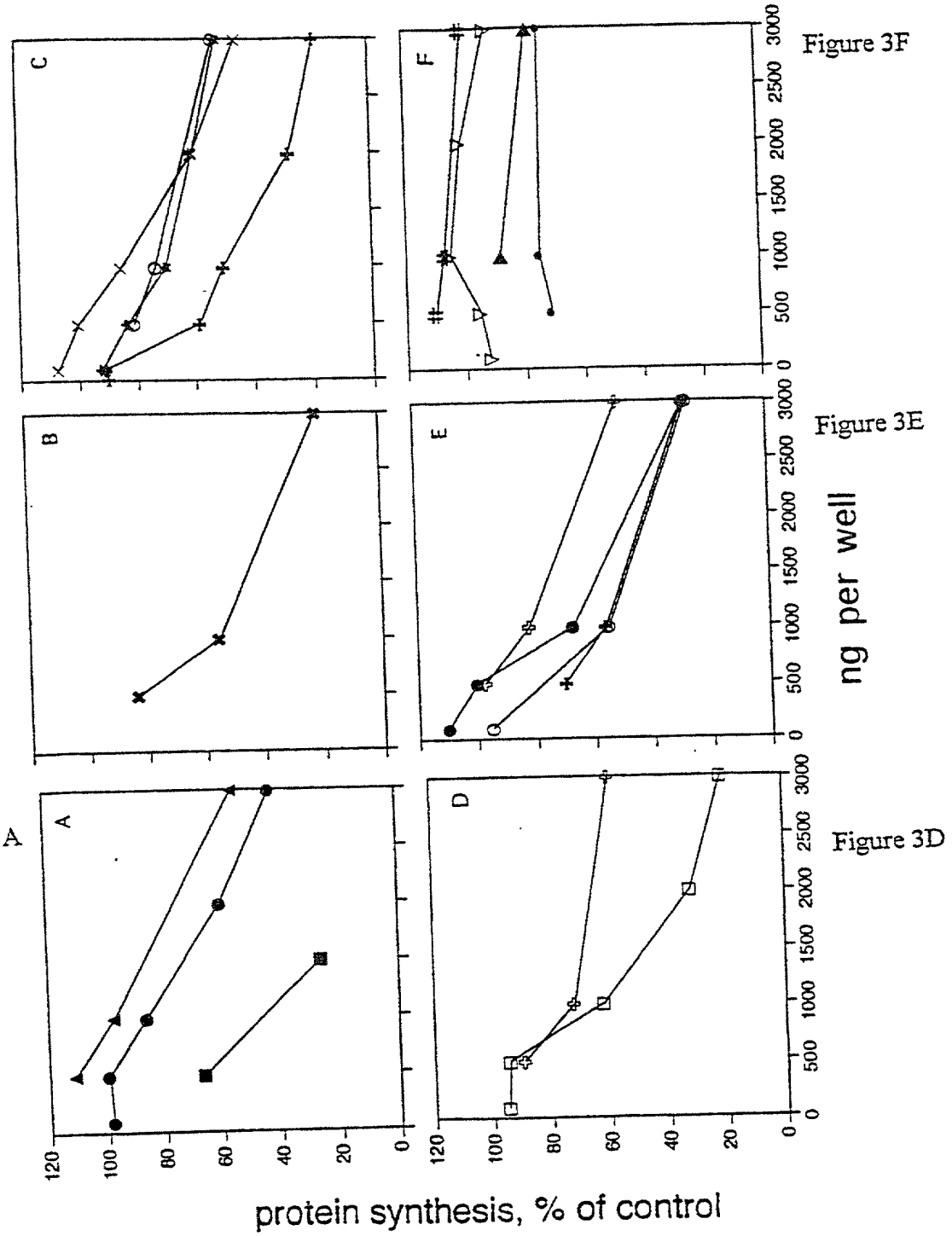


Figure 3A

Figure 3B

Figure 3C

Figure 3D

Figure 3E

Figure 3F

Figure 4A

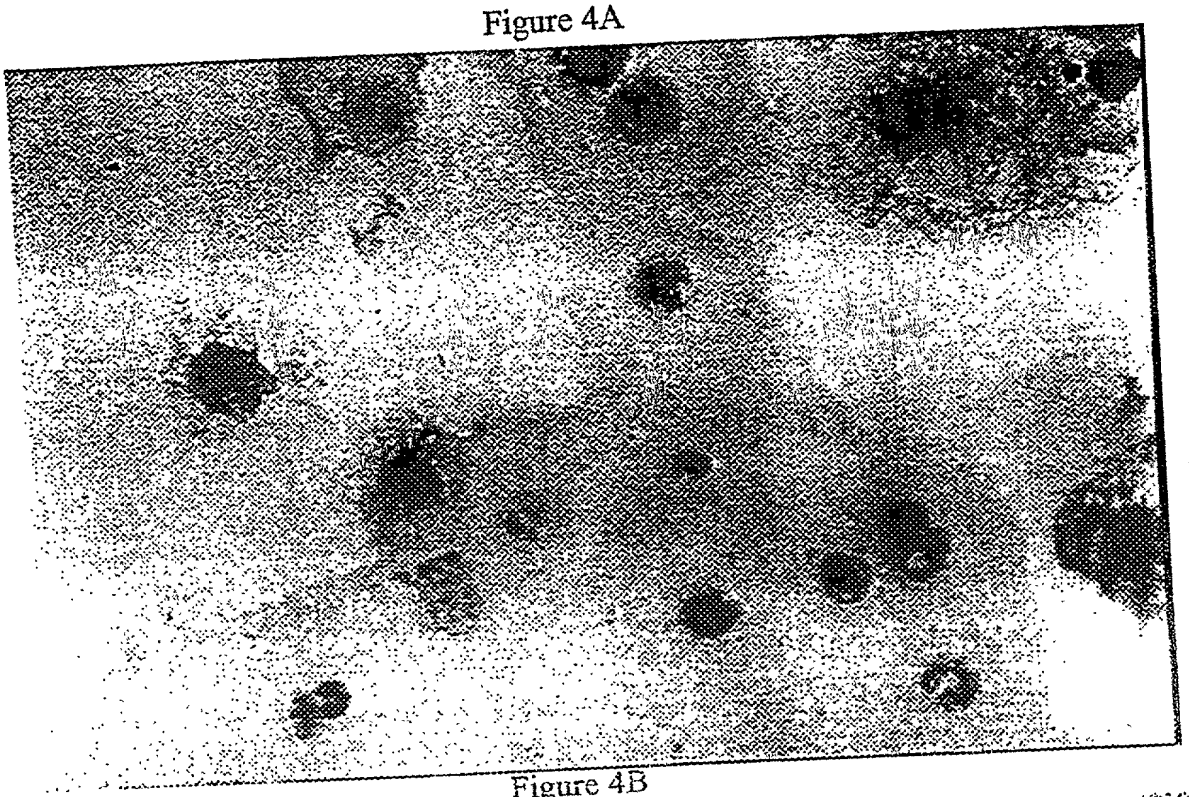


Figure 4B

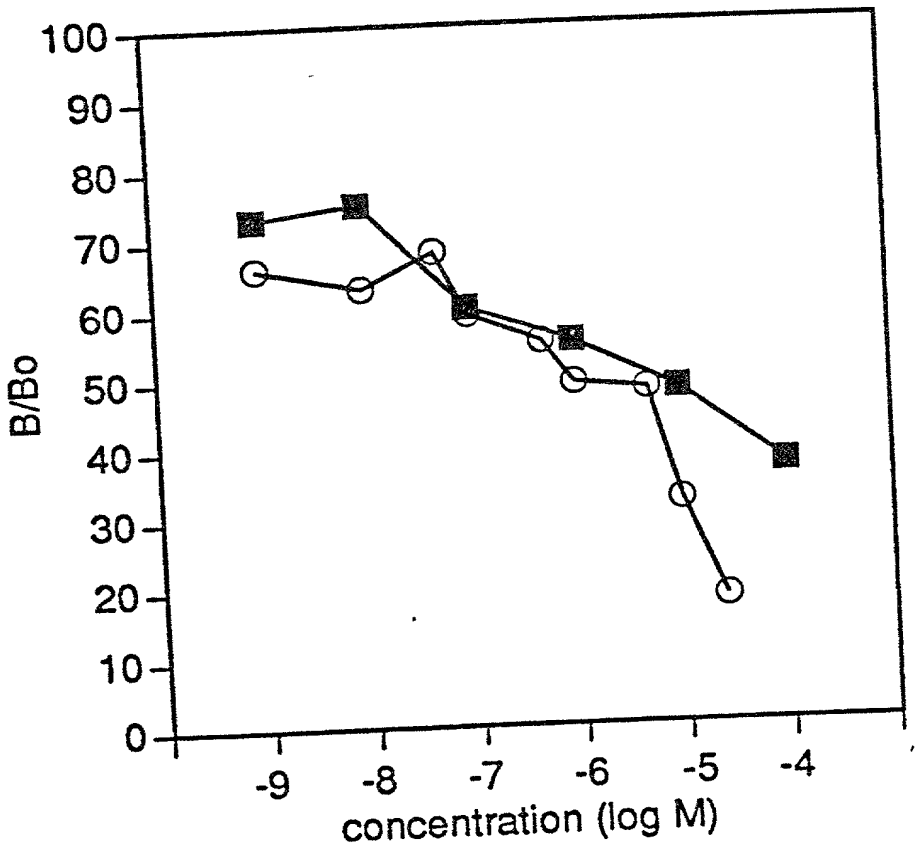


Figure 5

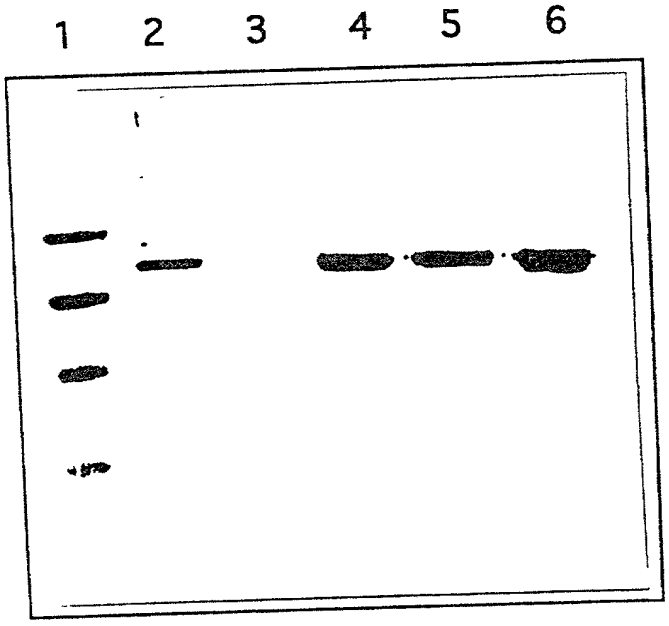


Figure 6

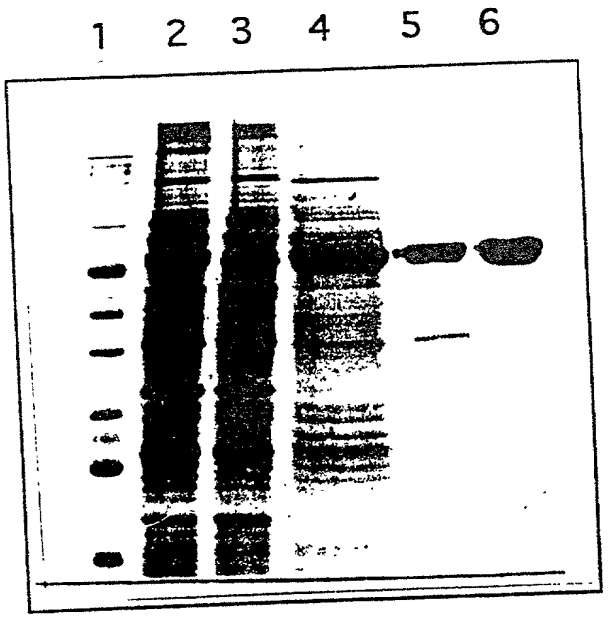


Figure 7

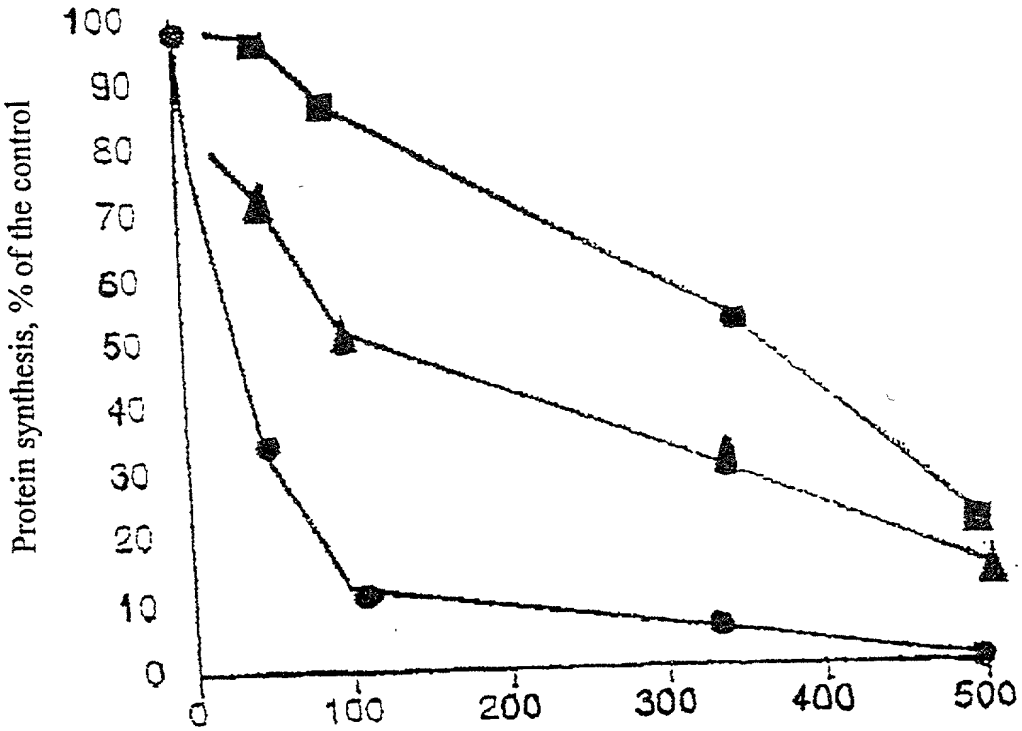

Protein synthesis, % of the control
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500

Figure 8

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Application(s)	Attorney's Docket Number 1268-073

As below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHIMERIC TOXINS FOR TARGETED THERAPY

the specification of which:

[] is attached hereto.

[X] was filed as United States application Serial No. 09/147,346

on December 4, 1998

and was amended on December 4, 1998 (if applicable).

[X] was filed as PCT international application Number PCT/IL97/00180

on 4 June 1997

and was amended under PCT Article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or Section 365(b) of any foreign and/or international application(s) for patent or inventor's certificate or Section 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (If PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
Israel	118570	4 June 1996	[XX] Yes [] No
			[] Yes [] No
			[] Yes [] No
			[] Yes [] No
			[] Yes [] No

I hereby claim the benefit under 35 USC §119(e) of any United States provisional application(s) listed below.

PRIOR PROVISIONAL APPLICATION(S):

Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this applicati is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge th duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the fil date of the prior application(s) and the national or PCT International filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (Check One)		
U.S. Application Number	U.S. Filing Date	Patented	Pending	Abandoned

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT Application No.	PCT Filing Date	U.S. Serial Numbers Assigned *(if any)*			

POWER OF ATTORNEY: As named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Allan M. Lowe, Reg. No. 19,641; Israel Gopstein, Reg. No. 27,333; Benjamin J. Hauptman, Reg. No. 29,310; Kenneth M. Berner, Reg. No. 37,093 and Michael G. Gilman, Reg. No. 19,114..

Send Correspondence to:	Direct Telephone Calls to: *(name and telephone number)*
LOWE HAUPTMAN GOPSTEIN GILMAN & BERNER, LLP 1700 Diagonal Road, Suite 310 Alexandria, Virginia 22314	(703) 684-1111

201	Full Name of Inventor	Family Name YARKONI	First Given Name Shai	Second Given Name
	Residence and Citizenship	City Kfar Saba IL	State or Foreign Country Israel	Country of Citizenship Israel
	Post Office Address	Post Office Address Hatamed Heh Street 33	City Kfar Saba	State & Zip Code/Country 44395 Israel
202	Full Name of Inventor	Family Name NECHUSHTAN	First Given Name Amotz	Second Given Name
	Residence and Citizenship	City Ramat Hasharon IL	State or Foreign Country Israel	Country of Citizenship Israel
	Post Office Address	Post Office Address Habanim Street 31	City Ramat Hasharon	State & Zip Code/Country 47223 Israel
203	Full Name of Inventor	Family Name LORBERBOUM-GALSKI	First Given Name Haya	Second Given Name
	Residence and Citizenship	City Jerusalem IL	State or Foreign Country Israel	Country of Citizenship Israel
	Post Office Address	Post Office Address Bar Kochva Street 72/3	City Jerusalem	State & Zip Code/Country 97875 Israel

I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of application or any patent issuing thereon.

Signature of Inventor 201:	Signature of Inventor 202:	Signature of Inventor 203:
Yarkoni Shai	Amotz Nechushtan	Lorberboum-Galski H.
Date Feb 10, 99	Date Feb. 1. 99	Date January 25, 1999

ADDITIONAL INVENTOR

204	Full Name of Inventor	Family Name MARIANOVSKY	First Given Name Irina	Second Given Name
	Residence and Citizenship	City Jerusalem TLX	State or Foreign Country Israel	Country of Citizenship Israel
	Post Office Address	Post Office Address Medical School of Hadasa, Dept. of Cellular Biochemistry, Ein Karem	City Jerusalem	State & Zip Code/Country 91120 Israel
205	Full Name of Inventor	Family Name	First Given Name	Second Given Name
	Residence and Citizenship	City	State or Foreign Country	Country of Citizenship
	Post Office Address	Post Office Address	City	State & Zip Code/Country
206	Full Name of Inventor	Family Name	First Given Name	Second Given Name
	Residence and Citizenship	City	State or Foreign Country	Country of Citizenship
	Post Office Address	Post Office Address	City	State & Zip Code/Country

I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of application or any patent issuing thereon.

Signature of Inventor 204:	Signature of Inventor 205:	Signature of Inventor 206:
Date Feb. 10. 99	Date	Date